Innovations

Lead compound, phone home

IRORI

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Before the advent of combinatorial chemistry a chemist needed only a felt-tipped pen to keep track of her compounds. But with the synthesis of huge libraries of compounds now common, a system for labeling newly synthesized compounds is enough to form the basis of a company.

One such company is IRORI (La Jolla, California), which sells capsules of solid-phase synthesis resin with embedded radiofrequency tags, and sorting machines that can read the code stored on the tags (Figure 1). Synthesis software directs the sorting of the capsules, and keeps track of which synthesis history is associated with which code.

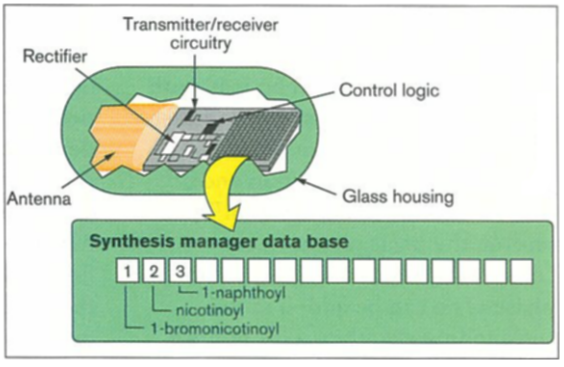
A new use for an old component

Radiofrequency (rf) tags are far from new. They are found in everything from building-entrance keys to injectable pet ID tags, and the IRORI tags are not spectacularly different in terms of design or communications capability. What was new in 1995, when Michael Nova and Andrew Senyei of IRORI and K.C. Nicolaou (of Scripps Research Institute, La Jolla, California, and an Editor of this journal) reported the construction of their first rf tags, was the application of rf technology to combinatorial chemistry.

In combinatorial chemistry, one of a large number of possible subunits is added to each of several variable sites on a molecule. Conceptually, the simplest way to make these compounds is by parallel synthesis, using a large matrix of individual reactions. But if every possible chemical group is to be combined with every other, a lot of time can be saved by using the split/pool method. For example, the three reaction products A, B and C can be combined, mixed, and then split into four mixtures to be reacted with components D, E, F and G to produce 12 different compounds. Further rounds of splitting and pooling give $X \times Y \times Z$ products from only $X + Y + Z$ reactions. A series of four reactions, each using ten different components, should result in 10,000 different products.

The trick with split/pool is to keep track of all those compounds. The resin beads used for solid-phase synthesis solve one problem: they keep many molecules of the same compound in one place. But the identity of the compound on the bead must still be decoded. Methods for directly determining the structure of compounds on a single bead are still in their infancy, so a tag or code that details the synthesis history of the bead is the next best option.

Figure 1



The IRORI radiofrequency tag. An antenna receives energy to power up the device, with the rectifier converting the radiofrequency pulse to direct circuit current. The chip code is then read, and communicated to the external reader using a radiofrequency pulse generated by the antenna. This code tells the synthesis manager software where to send the capsule for the next synthesis step. At the end of the synthesis, the code is read one last time. The identity of the code is compared to a database to derive the synthesis history of the capsule, and thus the expected structure of the synthesized compound, which is on the matrix surrounding the tag. Image courtesy of IRORI.

The IRORI system

In their original paper in *Angewandte Chemie*, the IRORI chemists wrote a code to each microreactor (containing resin and rf tag) whenever a synthesis step occurred. The more recent commercial version assigns a code at the outset to a proposed final compound. A sorter reads the code at the conclusion of every step and sends the microreactor to the appropriate reaction chamber.

Although the new system sounds more cumbersome, it results in huge savings in the numbers of reactions. To ensure that most compounds will be made in a given split/pool reaction sequence, the chemist must include at least ten times more beads than prospective products. But with the 'directed sorting' used by IRORI, one microreactor per compound is enough. This means less chemicals, less resin, and less decoding at the end. Rf tags are re-usable, but keeping their numbers down in any one experiment is also important, as they go for $8.50 a pop.

The software to direct the synthesis, and the sorter to redistribute up to 10,000 microreactors in 10 hours, are also not cheap, selling for a total of ~$100,000. Is all this technology really necessary? "I remember when I first saw [the rf tag system] I thought it was cute, but that it looked like a two dollar solution to a two cent problem," admits Tony Czarnik, now Vice-President for Chemistry at IRORI. "But it is actually a very elegant solution to a problem that there aren't obvious alternatives to." According to Matt Francis of Harvard University (Cambridge, Massachusetts): "It lets you make something with the benefits of a parallel library, with discrete compounds made in significant amounts, but using the efficiency of split/pool synthesis."

The unique tag is encased in glass, so it is "inert to all the slings and arrows that a synthetic chemist can throw at it," says Czarnik. "And the code," he says, "can be read with a very reliable, very fast method that doesn't depend on orientation."

The tags are enclosed in one of two containers. In a MicroKan™ reactor, the tag is placed in the center of the vessel and any solid-phase resin can be added to the surrounding chamber. This offers a familiar substrate for synthesis, but involves some labor in setting up the microreactors. The MicroTube™ reactors are ready to go, with synthesis occurring on a polymer tube that surrounds the tag. Chemistry happens on the surface of the polymer, whereas reactions with resin occur primarily inside the resin bead. As a result, some synthesis condition need to be modified for the MicroTube™ reactor. In both cases, the amount of product is in the tens of milligrams: a large amount in the world of combinatorial chemistry.

The sorter can scan and distribute up to 10,000 microreactors in one 10-hour run. This speed, and the cost of the reactors, constrain the size of the libraries. "It's the medium-sized libraries we think the IRORI system will be best at," says Francis. Libraries of less than one hundred can be done by manual parallel synthesis, while libraries of more than ~20,000 may be too expensive and take too long to make with the current technology. "To get to libraries of more than 20,000," says Czarnik, "you probably need to make less compound" by reducing the size of the reactors. And, he adds, "the sorting has to get faster."

IRORI is working on a faster sorter, but reducing the size of the rf tag may be difficult. "It's not clear that there is a direct way to make the tags smaller, as the antenna [which takes up ~90% of the volume of the tag] has to be big enough to power itself up." Solutions to both the size and cost problems are under development at IRORI.

According to some chemists, the big libraries that demand smaller and cheaper tags may not even be desirable. "There is as much range of opinion [on library size] as there are people," says Czarnik. "There are devout believers in spending as much time thinking about what you should make as possible, and then never making more than a few hundred compounds. Then there are those who say, 'I would like to know exactly what to make, but I would also like to know the face of God, so perhaps we should make lots of compounds.'"

The many alternatives

Almost coincident with the IRORI paper was a report in the *Journal of the American Chemical Society* from researchers at Ontogen Corporation (Carlsbad, California). They proposed rf tags that were essentially the same as the IRORI tags, while their synthetic strategy used directed sorting. More recently, however, Ontogen has concentrated on building large automated machines for parallel solid-phase synthesis.

A research group at Pfizer, Inc. (Groton, Connecticut) led by Eric Roskamp has reported a simple solution to the tagging problem: numbering the vessels. Roskamp used a heated printing press and graphite ink to imprint a number on his plastic microreactors. A video camera and optical character recognition software could scan through ~1000 microreactors in a day. "In the worst case, if you really rub on it, the black will wear off," he says. "But you can still read [the imprint] by eye." He will not give details of the new version of this proprietary machine, except to say that it "does the same things, but faster and more efficiently."

An additional tagging system for IRORI scientists may incorporate the 2D bar codes they described late last year in *Angewandte Chemie*. The patterns of dark and light squares are laser-etched onto a ceramic surface, and then read by a camera.

The predecessors of these high-tech solutions include oligonucleotides and peptides, with a single base or amino acid added at each step of synthesis. The chemistry of these tags is not, however, compatible with many reactions used in small molecule synthesis. This led Clark Still of Columbia University to develop sets of small, halogen-substituted aromatics that could be used as tags (technology now licensed to Pharmacopeia, Inc. (Princeton, New Jersey)), while researchers at Affymax (Palo Alto, California) devised secondary amine tags.

Some methods bypass tags altogether. Deconvolution identifies a hit compound by deducing the preferred component at each position in turn. But this method relies on both extensive resynthesis and the testing of mixtures of compounds, and it identifies only one hit compound.

Perhaps the most widespread method, even in solid-phase synthesis, is to keep each reaction separate by spatial coding. Steps like washing and rinsing can be pooled, but many of the time-saving advantages of split/pool are lost. The method remains popular because it is not a proprietary technology.

Solid-phase synthesis allows a chemist to drive reactions to completion with excess reagents, and to isolate products free from contaminants. Chemists are working on automated purification schemes for the solution phase, but even without these methods the more familiar techniques of solution-phase synthesis remain popular. "The biggest barrier is a cultural one," says Czarnik, but he is confident that barrier will come down. "We are placing a sizable bet that split/pool is an irresistible force," says Czarnik, "and that people will see this as the direction to move in."

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